# Autoantibodies against the Multicatalytic Proteinase in Patients with Systemic Lupus Erythematosus

By Joaquín Arribas,* Maria Luz Rodríguez,* Rita Alvarez-Do Forno,‡ and José G. Castaño*

*From *Departamento de Bioquímica e Instituto de Investigaciones Biomédicas del CSIC, Facultad de Medicina de la UAM; and ‡Servicio de Inmunologia Ciudad Sanitaria La Paz, 28029 Madrid, Spain*

## Summary

Sera from patients with systemic lupus erythematosus contain specific autoantibodies directed against different polypeptide components of the multicatalytic proteinase (also known as proteasome or prosome). These human autoantibodies, in contrast to polyclonal antibodies obtained in rabbits against the purified enzyme, recognize highly conserved epitopes of the multicatalytic proteinase polypeptides from yeast to human.

Multicatalytic proteinase (MCP),[1] also known as proteasome or prosome, is a recently described high molecular weight proteinase composed of several polypeptides ranging in size between 20 and 35 kD and shown to be identical to a previously described 19S particle present in all eukaryotes having a characteristic cylindrical shape formed by four stacked rings (1–4). The enzyme responsible for the processing of the 5′ end of pretRNAs in *X. laevis* oocytes (5) and aminoacyltransferase I (6) has been shown to be associated with this cylindrical particle. Here, we report, for the first time, the presence with relative high frequency of antibodies against the polypeptide components of this multicatalytic proteinase in the sera of patients with SLE and the high degree of conservation of the epitopes recognized by these autoantibodies during evolution from yeast to human.

## Material and Methods

*Screening of Patient's Sera.* MCP from yeast, *X. laevis* ovaries, mouse EAC, and human erythrocytes was purified essentially as described (7) and judged 90–95% homogenous by SDS-PAGE. The MCP was subjected to one- (8) or two-dimensional (9) electrophoretic analysis, and either stained with Coomassie brilliant blue or silver, respectively, or Western blotted (10) and probed with sera of patients with autoimmune diseases at 1/100 dilution, as described (11). The blots were developed with peroxidase-labeled goat anti-human antibodies (Cappel Laboratories, Cochranville, PA). Purified MCP was also probed against sera of these patients by counterimmune electrophoresis (12).

*Affinity Purification of Antibodies against the MCP Polypeptides.* The antibodies against MCP present in patient sera were affinity purified essentially as described (13). Briefly, purified MCP from Ehrlich ascites cells (EAC) was subjected to SDS-PAGE, Western blotted, and incubated with 0.5 ml of patient serum; the localization of anti-MCP antibodies was assessed by developing vertical stripes cut from the sides with goat peroxidase-labeled anti-human antibodies. The band containing anti-MCP autoantibodies was washed with 0.2 M Gly, pH 2.4, to elute the bound antibodies. This solution was immediately neutralized after elution with 0.5 M NaOH and diluted threefold with TBS (50 mM Tris-Cl, pH 7.4, 150 mM NaCl). Finally, the diluted and neutralized solution was dialyzed against TBS, concentrated to 0.5 ml on a centrikon ultrafilter, and used for immunoblot.

*Obtention of Rabbit Polyclonal Antibodies against Rat MCP.* Rabbit anti-rat MCP polyclonal antibodies were obtained by subcutaneous injection of 100 μg of purified rat liver MCP emulsified with CFA (first injection) and three more injections (100 μg each) with IFA at 1-wk intervals. Serum was obtained 2 wk after the last injection; the titer of the rabbit antiserum obtained was 1/1,000.

## Results and Discussion

We initially purified the MCP from mouse EAC and used the purified enzyme for a screening by immunoblot of sera of patients with autoimmune diseases that have the following clinical diagnoses: 43 patients had SLE, four had dermatomyositis, five had polymyositis, four had sclerodermia, and three have been diagnosed with Sjogren syndrome. The presence of antibodies against the polypeptides components of the EAC MCP was detected in 15 (35%) of the group of patients that had SLE with titers that varied from 1/50 to 1/200. None of the other sera tested, including CDC reference sera against U1 snRNP, Sm, La, and Ro, were positive, and a pool of 100 normal subjects was also negative (data not shown). An example of two of the positive sera is presented in Fig. 1 *A*. The antibodies present in the serum of these patients also recognized, as expected, the homologous human erythrocyte

[1] *Abbreviations used in this paper:* EAC, Ehrlich ascites cells; MCP, multicatalytic proteinase.


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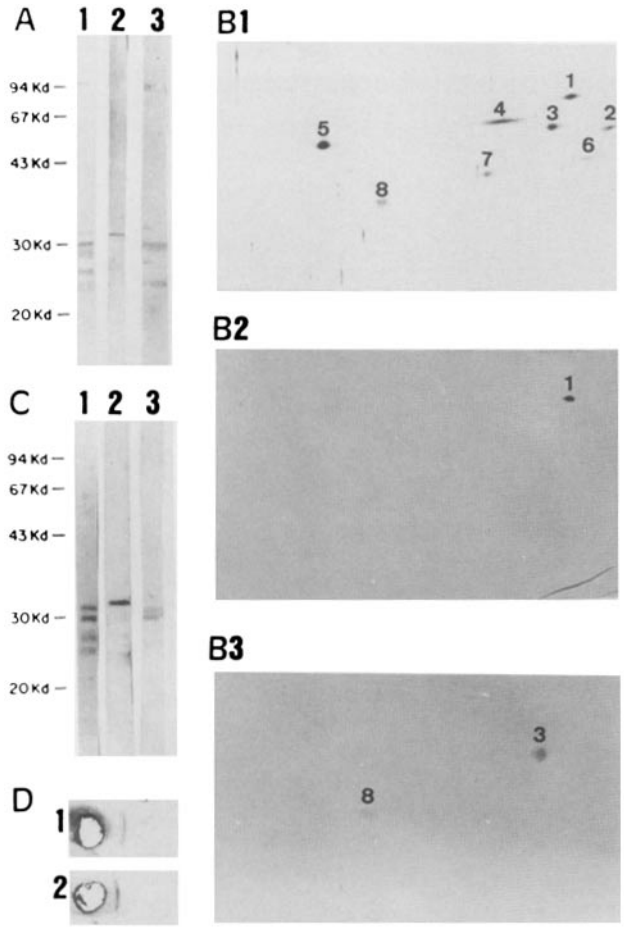


**Figure 1.** Characterization of autoantibodies against multicatalytic proteinase in sera of patients with SLE. (*A*) Lane *1*, One-dimensional SDS-PAGE of the purified enzyme from EAC stained with Coomassie brilliant blue; lanes *2* and *3*, Western blot probed with S-122 and R4392 sera from SLE patients, respectively. (*B*) EAC multicatalytic proteinase proteins analyzed by two-dimensional gel electrophoresis. Lane *1*, silver-stained gel; lane *2*, two-dimensional blot probed with S-122 serum; lane *3*, two-dimensional blot probed with R4392 serum; (*C*) SDS-PAGE of MCP from human erythrocytes stained with Coomassie brilliant blue (lane *1*) or Western blotted and probed with S-122 serum (lane *2*) or R4392 serum (lane *3*). (*D*) Counterimmune electrophoresis of MCP from EAC against S122 serum (lane *1*) or R4392 serum (lane *2*). Antigen (20 μg) was placed in the left wells, and sera were placed in the right wells (10 μl). After electrophoresis and washing, the strips were stained with Coomassie blue.

MCP (Fig. 1 *C*). To further characterize the polypeptides of the MCP to which these autoantibodies were directed, two-dimensional immunoblotting of EAC MCP was performed. Results of this analysis for the sera of the two patients shown in *A* are presented in Fig. 1 *B*. From several different preparations of EAC MCP, eight polypeptides were consistently observed by two-dimensional analysis and are numbered 1–8 (Fig. 1 *B*, lane *1*). The serum of patient S122 mainly recognized polypeptide 1 (Fig. 1 *B*, lane *2*), and the sera R4392 recognized polypeptides 3 and 8 (Fig. 1 *B*, lane *3*). In Fig. 1 *D*, the results of counterimmune electrophoresis against the native MCP of the two sera presented above are shown. The formation of a precipitation line indicates that the autoantibodies present in those sera are able to recognize the native MCP and to form immunoprecipitable complexes. Autoantibodies directed against each of the polypeptides of the MCP have been found, but individual serum recognizes one or two of those polypeptides, and no serum has been found to recognize the whole set of polypeptides of the MCP or to inhibit its proteinase activity when added directly to MCP. These results indicate that the immune response against individual MCP proteins differs from patient to patient and either some of the protein components of the MCP share common epitopes or, alternatively, some of the polypeptide bands observed may be derived from others by autocleavage due to the proteolytic activity of MCP (14).

When different amounts of the 15 different positive sera (10–100 μl) from the patients, either total serum or affinity purified (see below), were incubated in the presence of 2 μg of the purified human erythrocyte MCP, there was no inhibition (100% of the enzymatic activity either in the presence or in the absence of antibody) of the proteolytic activity of the MCP as assayed with three fluorescent model substrates (ARR, for trypsin-like activity, LLVY for chymotrypsin-like activity, and LLE for peptidylglutamyl-peptide hydrolytic activity; see reference 7 for details of the assay procedure). Similarly, 80–90% of the enzymatic activity was recovered in the pellet of the immunoprecipitation of the antigen antibody complex with protein A–Sepharose. These results indicate that none of the autoantibodies we have detected are blocking antibodies either in solution or after immunoprecipitation of the immune antigen-antibody complex with protein A–Sepharose.

SLE patients frequently have more than one type of autoantibodies (15), so it could be possible that antibodies detected in the serum of these patients were directed against other antigens showing simple crossreaction with the particle polypeptides. To clarify this point, the antibodies bound to transferred particle polypeptides were eluted and tested for specificity. Proteins from the different fractions obtained through the purification of EAC MCP were separated by SDS-PAGE, transferred, and immunoblots were done with R4392 serum unpurified or affinity purified as indicated above. The results of this experiment (presented in Fig. 2) show that, as expected, the unpurified R4392 serum recognize several polypeptides in fractions from early steps of purification apart of the MCP polypeptides, but after purification, only the polypeptide components of the MCP are recognized in all fractions. This result indicates that genuine specific antibodies against MCP polypeptides are present in this patient. Similar results have been obtained with five other positive sera (data not shown).

As already shown above, the autoantibodies detected recognize both human and mouse MCP polypeptides, indicating a certain degree of conservation of the recognized epitopes. To further explore this conservation, the MCP from yeast, *X. laevis* ovaries, rat liver, and human erythrocytes were purified and their protein components were probed by immunoblots with human serum. Fig. 3 shows that R4392 serum (*C*) and

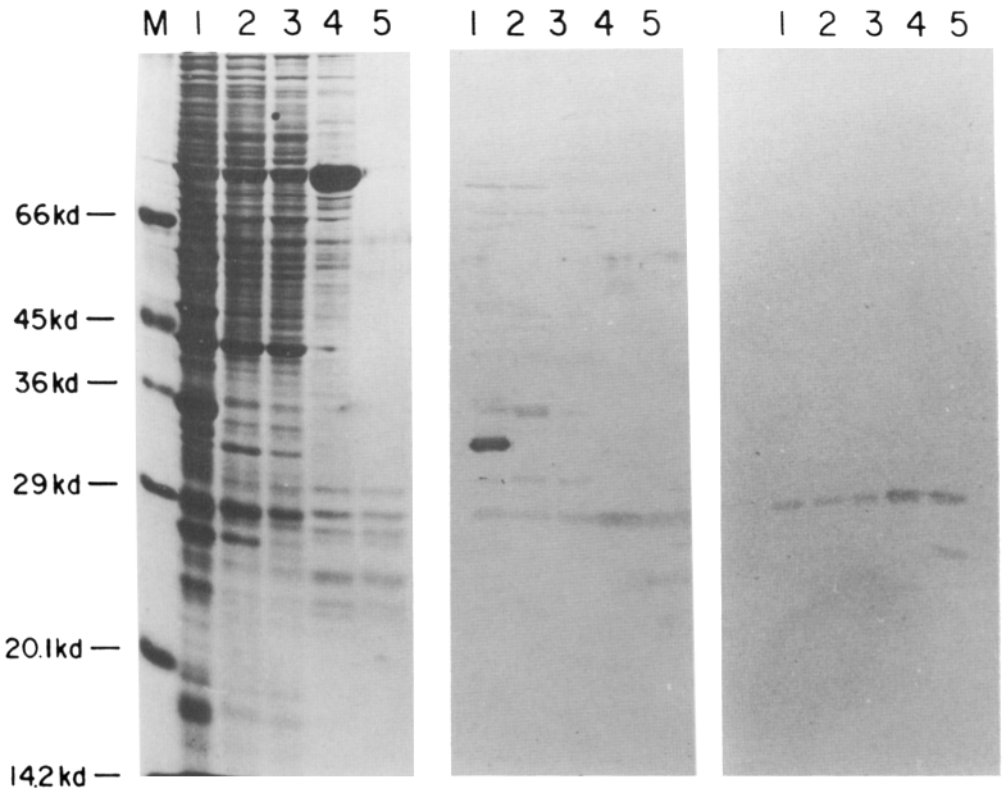


**Figure 2.** Specific anti-MCP antibodies are actually present in the serum of SLE patients. SDS-PAGE of the different steps of the purification of MCP from EAC (see reference 7): lanes *1*, high-speed supernant of the homogenate (100,000 *g*, 60 min); lanes *2*, DEAE-cellulose; lanes *3*, hydroxyapatite; lanes *4*, DEAE-AffiGel blue; lanes *5*, glycerol gradient. (*Left panel*) Gel stained with Coomassie Blue; (*center panel*) immunoblot of the same fractions with R4392 serum; (*right panel*) immunoblot of the same fractions probed with anti-MCP antibodies affinity purified from R4392 serum.

R5192 serum (*D*) recognize similar polypeptides in all four MCP, similar results have been obtained with four other human sera (data not shown). These results clearly point out the high degree of conservation of the epitopes recognized by the human autoantibodies. In contrast, a rabbit polyclonal antibody obtained against the native MCP from rat liver (Fig. 3 *B*) recognizes almost the whole set of polypeptides of the MCP from rat liver and shows almost no crossreaction with yeast MCP polypeptides. Similar results have been reported with polyclonal antibodies obtained against MCP from one eukaryotic species showing poor crossreactivity with MCP from other species (16).

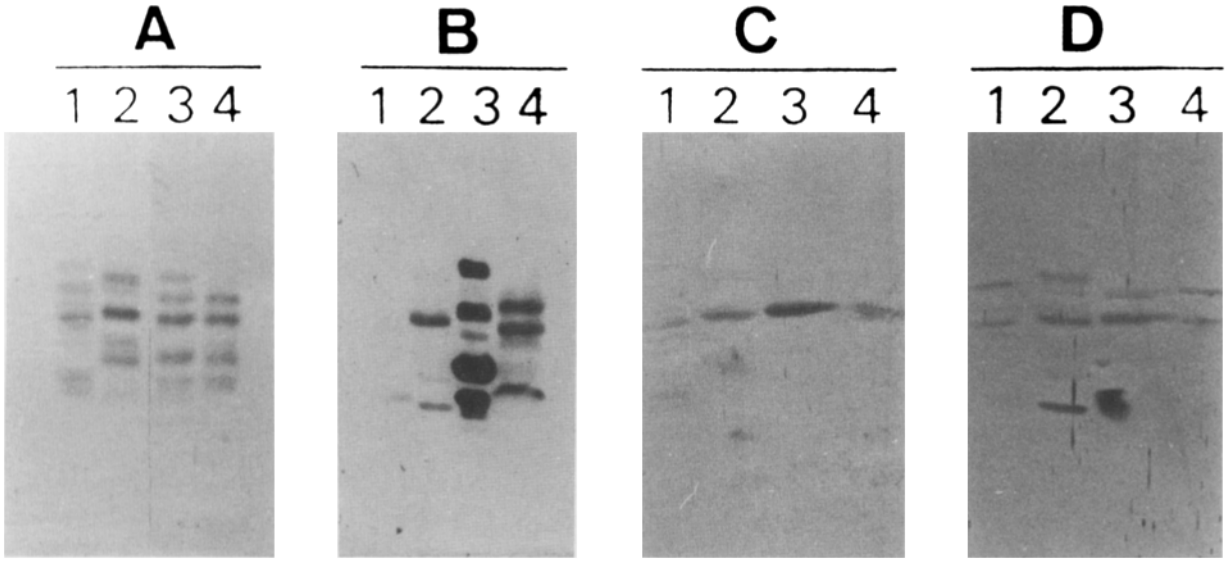


**Figure 3.** Conservation of the epitopes of MCP recognized by serum of patients with SLE. MCP was purified from different sources essentially as described (7) and analyzed by SDS-PAGE yeast (lane *1*), *X. laevis* ovaries (lane *2*), rat liver (lane *3*), and human erythrocytes (lane *4*) and either stained with Coomassie blue (*A*) or transferred and immunoblotted with rabbit antiserum (*B*), human S-122 serum (*C*), and R4392 serum (*D*) at 1/100 dilution. The blots were developed with corresponding peroxidase-labeled goat anti-rabbit or goat anti-human antibodies.

Evidence has been presented for the presence of specific antibodies against the polypeptide components of the multicatalytic proteinase in 35% of patients with SLE. This frequency is within the range of autoantibodies against snRNPs (15). Increasing evidence indicates that the autoimmune response is antigen driven, but for most of the autoantigens characterized in SLE patients, except for DNA, no evidence has been presented of circulating antigens that can form complexes with the autoantibodies (15). The MCP is a protease with unknown function in the cell. Within the cell, it seems to be located both in the nucleus and the cytoplasm (2, 17–21), and in early descriptions (22), was also found in the culture media of tumor cells. The fact that MCP is also present in circulating nonnucleated cells, like the erythrocytes (21, present report), and in platelets (Arribas, J., and J. G. Castaño, unpublished results), makes its presence in plasma very likely and available to form immune complexes in patients that have autoantibodies. As the autoantibodies detected do not inhibit the proteinase activity of the MCP, these immune complexes could be involved, directly or indirectly, in attacking membrane proteins producing cell damage as a part of the complex response that eventually produces the pathological lesions observed in different tissues of autoimmune patients.

Another interesting aspect is that the epitopes of the MCP polypeptides recognized by the human autoantibodies are highly conserved from yeast to human. This is in contrast to the natural response observed against MCP antigen in rabbits, suggesting a mono- or oligoclonal B cell activation together with an unusual antigen presentation in SLE patients or a selective immune response due to a particular infectious (viral, bacterial, or fungi) antigen similar to MCP. It has not been possible to identify a proteinase like MCP in eubacteria (*Escherichia coli*), but a high molecular weight proteinase with a similar cylindrical structure as the MCP has been found in archaebacteria (23), and a similar structure by electron microscopy has been described as part of the protective antigen of *Bordetella pertussis* (24). A stronger suggestion that *E. coli* may have a protease related to one of the components of the MCP is the report that antibodies against the proteinase subunit (P subunit) of Ti protease from *E. coli* crossreact with one of the polypeptides of yeast and rat liver MCP (25), and more recently, it has been shown that the purified C1p protein has a structure (electron microscopy) similar to MCP and is well conserved to eukaryotes (26, 27). If some of the autoantibodies identified against the MCP polypeptides could also recognize those bacterial proteases, this may indicate that immunological memory of bacterial or fungal infection may lead in certain individuals to the autoimmune response. In this sense, MCP from yeast and their corresponding bacterial homologues would play a role, in certain patients with SLE, similar to mycobacterial hsp60 in patients with rheumatoid arthritis (28 and references therein).

The detection of these newly described autoantibodies against the multicatalytic proteinase may be important for the clinical diagnoses and prognoses of certain patients with SLE. Further research will clarify the role of these autoantibodies in the pathogenesis and etiology of SLE and their possible existence in other autoimmune diseases.

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Address correspondence to José G. Castaño, Dpto. de Bioquímica e Ins. Investigaciones Biomédicas, Facultad de Medicina de la UAM, Arzobispo Morcillo, 4, 28029 Madrid, Spain.

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